

SUPPORTING INFORMATION

Diclofenac identified as a kynurenone 3-monooxygenase binder and inhibitor by molecular similarity techniques.

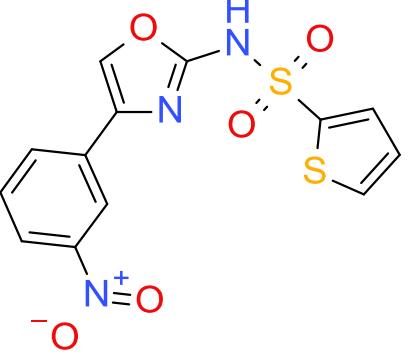
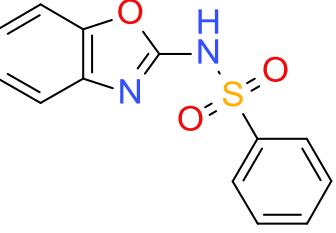
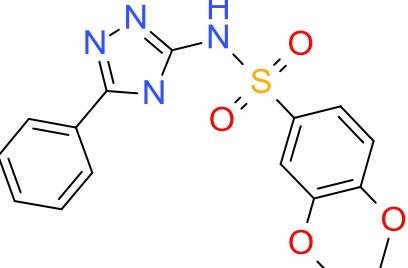
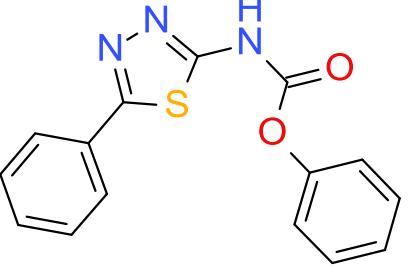
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Table S1 – Known KMO inhibitors used as input to molecular similarity techniques.

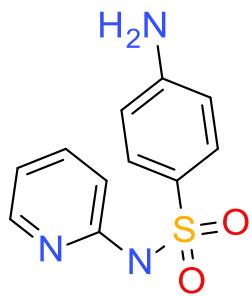
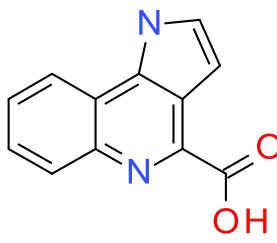
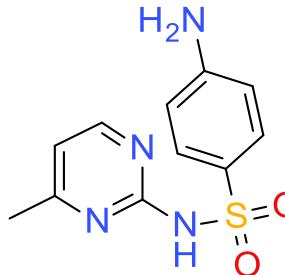
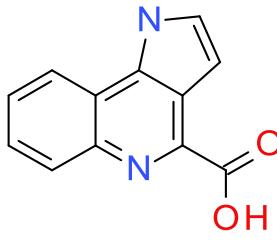
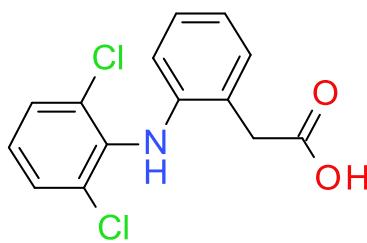
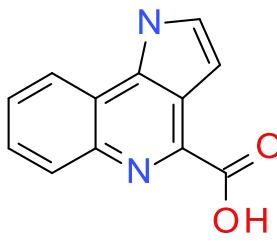
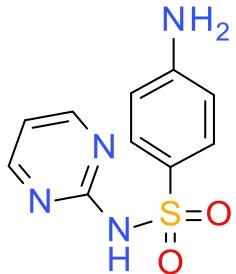
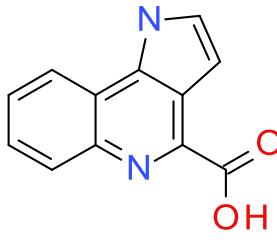
2D	Identifier, Name, SMILES, (Note), Reference
	<ul style="list-style-type: none"> • Pharmacia WO199805660 Example • 1H-pyrrolo[3,2-c]quinoline-4-carboxylic acid • O=C(O)c1nc2ccccc2c2[nH]cccc12 • IC₅₀ unknown, but 3 derivatives of this core structure have IC₅₀s < 100 μM in Rat KMO. • Pevarello, P., Heidemperger, F., Della, T. A., Varasi, M., and Speciale, C. (1998) Pyrrolo(3,2-c) quinoline derivatives, WO 1998005660 A1
	<ul style="list-style-type: none"> • RocheCompound16 • 3,4-dimethoxy-N-[4-(3-nitrophenyl)thiazol-2-yl]benzenesulfonamide • O=S(=O)(Nc1nc(cs1)c1cccc(c1)N(=O)O)c1cc(OC)c(O)cc1 • Rat KMO IC₅₀: 37nM • Röver, S., Cesura, A. M., Huguenin, P., Kettler, R., and Szente, A. (1997) Synthesis and biochemical evaluation of N-(4-phenylthiazol-2-yl) benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase, <i>Journal of medicinal chemistry</i> 40, 4378-4385
	<ul style="list-style-type: none"> • RocheCompound19 • N-[4-[2-fluoro-6-(trifluoromethyl)phenyl]thiazol-2-yl]-3,4-dimethoxy-benzenesulfonamide • O=S(=O)(Nc1nc(cs1)c1c(cccc1F)C(F)(F)F)c1cc(OC)c(O)cc1 • Rat KMO IC₅₀: 39nM • Röver, S., Cesura, A. M., Huguenin, P., Kettler, R., and Szente, A. (1997) Synthesis and biochemical evaluation of N-(4-phenylthiazol-2-yl) benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase, <i>Journal of medicinal chemistry</i> 40, 4378-4385
	<ul style="list-style-type: none"> • RocheCompound20 • 4-amino-N-[4-[2-fluoro-6-(trifluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide • O=S(=O)(Nc1nc(cs1)c1c(cccc1F)C(F)(F)F)c1ccc(N)cc1 • Rat KMO IC₅₀: 19nM • Röver, S., Cesura, A. M., Huguenin, P., Kettler, R., and Szente, A. (1997) Synthesis and biochemical evaluation of N-(4-phenylthiazol-2-yl) benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase, <i>Journal of medicinal chemistry</i> 40, 4378-4385

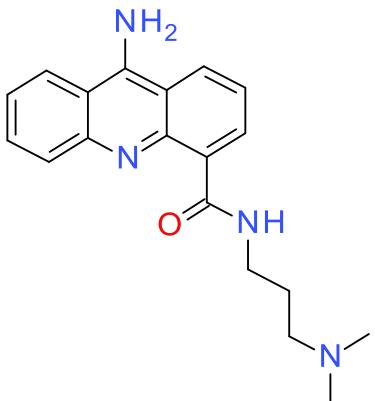
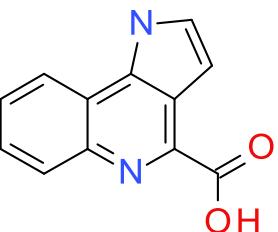
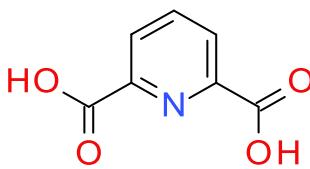
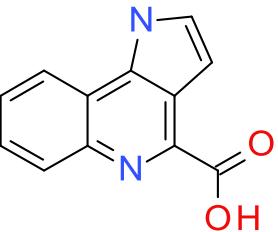
	<ul style="list-style-type: none"> • Pharmacia WO199928316 Example • N-[4-(3-nitrophenyl)oxazol-2-yl]thiophene-2-sulfonamide • O=S(=O)(Nc1nc(co1)c1cccc(c1)N(=O)O)c1cccs1 • Speciale, C., Toma, S., Heidempergher, F., Varasi, M., Pevarello, P. (1999) Thiophene-sulfonamide compounds, CA 2310054 A1
	<ul style="list-style-type: none"> • Pharmacia WO199928306 Example • N-(1,3-benzoxazol-2-yl)benzenesulfonamide • O=S(=O)(Nc1nc2cccc2o1)c1ccccc1 • Varasi, M., Pevarello, P., Heidempergher, F., Greco, F., Speciale, C. (1999) Benzenesulfonamide compounds, WO 1999028306 A1
	<ul style="list-style-type: none"> • UniversityOfMaryland WO2008022286 Example • 3,4-dimethoxy-N-(5-phenyl-4H-1,2,4-triazol-3-yl)benzenesulfonamide • COc1cc(ccc1OC)S(=O)(=O)Nc1[nH]c(nn1)c1ccccc1 • Muchowski, P. J., Muchowski, J. M., Schwarcz, R., Guidetti, P. (2008) Small molecule inhibitors of kynurenine-3-monooxygenase, WO 2008022286 A2
	<ul style="list-style-type: none"> • Pharmacia WO199928309 Example • phenyl N-(5-phenyl-1,3,4-thiadiazol-2-yl)carbamate • O=C(Oc1ccccc1)Nc1nnnc(s1)c1ccccc1 • Pevarello, P., Amici, R., Villa, M., Toma, S., Varasi, M. (1999) 1, 3, 4-thiadiazoles derivatives as kyn-oh inhibitors, WO1999028309 A1

	<ul style="list-style-type: none"> • Pharmacia WO199906375 Example 1 • (2R)-1-[(3aR)-3a,4-dihydrothiochromeno[4,3-c]pyrazol-3-yl]-3-amino-2-(3-phenylpropylamino)propan-1-one • NC[C@H](NCCCCc1ccccc1)C(=O)C1=NN=C2c3cccc3SC[C@H]12 • Paolo, P., Varasi, M., Heidemperger, F., Greco, F., Speciale, C. (1999) Fused heterocyclic compounds and their use as kynurenone-3-hydroxylase inhibitors, WO 1999006375 A1
	<ul style="list-style-type: none"> • Pharmacia WO199906375 Example 2 • (2R)-1-[(3aS,7aS)-1-methyl-3a,4,5,6,7,7a-hexahydroindazol-3-yl]-3-amino-2-(2-phenylethylamino)propan-1-one • NC[C@H](NC(Cc1ccccc1)C(=O)C1=NN(C)[C@H]2CC[C@H]12) • Paolo, P., Varasi, M., Heidemperger, F., Greco, F., Speciale, C. (1999) Fused heterocyclic compounds and their use as kynurenone-3-hydroxylase inhibitors, WO 1999006375 A1

Table S2 – Prioritized drugs for assay.

Query molecule for ligand based similarity analysis:	Query molecule compound was a UFSRAT similar to:	Tested?	Active?
 Quinaldic Acid	 Pharmacia WO199805660	✓	✗

 <p>Sulfapyridine</p>	 <p>Pharmacia WO199805660</p>	✓	✗
 <p>Sulfamerazine</p>	 <p>Pharmacia WO199805660</p>	✗	Unknown Not available
 <p>Diclofenac</p>	 <p>Pharmacia WO199805660</p>	✓	✓
 <p>sulfadiazine</p>	 <p>Pharmacia WO199805660</p>	✗	Unknown Not available

	 Pharmacia WO199805660	X	Unknown Not available
 Dipicolinic Acid	 Pharmacia WO199805660	✓	X

After purchase and assay of the above compounds, the X-ray crystal with PDB ID 4J36 became available. Docking of Quinaldic Acid, Sulfapyridine, Sulfamerazine, Sulfadiazine, Aminacrine and Dipicolinic acid using the same protocol used for Diclofenac showed no convincing binding mode.

Autodock vina docking protocol and parameters

The PDB file 4J36 was obtained from the RCSB protein databank. The structure was prepared in PyMol v1.4.1 (Schrödinger) by removal of the UPF 648 ligand with residue ID “1HR”. PDB2PQR¹ v1.8 was then used to prepare a pdbqt file representing the protein, run with command line options “--ff=PARSE --with-ph=7.4”.

A PDBQT file representing diclofenac was prepared by first generating a 3D SDF file using the conformation generation procedures proposed by Ebejer² and the structure protonated using OpenBabel³ v2.3.2. The Autodock⁴ tools program prepare_ligand4 was then used to generate a PDBQT file of the protonated diclofenac molecule. Autodock Vina⁵ v1.1.2 was then used to dock diclofenac to the prepared structure using the following parameters:

```
size_x = 17.00
size_y = 14.00
size_z = 18.00
center_x = 2.55
center_y = 48.00
center_z = 66.45
```

Table S3 – Targets from ChEMBL v23⁶ hit by molecules containing the pharmacia core scaffold (1H-pyrrolo[3,2-c]quinoline-4-carboxylic acid)

<u>ChEMBL Molecule ID</u>	<u>IC₅₀ (nM)</u>	<u>Target</u>
CHEMBL3589653	2600	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589663	1100	Dual specificity protein kinase CLK1
CHEMBL3589663	120	Cyclin-dependent kinase 9
CHEMBL3589663	32	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589660	40	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589660	45	Dual specificity protein kinase CLK4
CHEMBL3589660	210	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589660	31	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589664	320	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589664	210	Dual specificity protein kinase CLK4
CHEMBL3589664	2300	Dual-specificity tyrosine-phosphorylation regulated kinase 3
CHEMBL3589664	330	Dual specificity protein kinase CLK1
CHEMBL3589664	8000	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589665	25	Dual specificity protein kinase CLK1
CHEMBL3589665	23	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589665	68	Dual specificity protein kinase CLK4
CHEMBL3589665	390	Dual specificity protein kinase CLK2
CHEMBL3589665	1000	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589665	1000	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589665	1000	Cyclin-dependent kinase 9
CHEMBL3589665	33	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589673	39	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589673	210	Cyclin-dependent kinase 9
CHEMBL3589673	100	Dual specificity protein kinase CLK1
CHEMBL3589668	2300	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589668	940	Dual specificity protein kinase CLK1
CHEMBL3589669	2000	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589669	350	Dual specificity protein kinase CLK4
CHEMBL3589669	240	Dual specificity protein kinase CLK1
CHEMBL3589661	80	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589661	16	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589661	20	Dual-specificity tyrosine-phosphorylation regulated kinase 1A

CHEMBL3589661	32	Dual specificity protein kinase CLK1
CHEMBL3589661	160	Cyclin-dependent kinase 9
CHEMBL3589666	1400	Dual-specificity tyrosine-phosphorylation regulated kinase 3
CHEMBL3589666	230	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589666	18	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589666	42	Dual specificity protein kinase CLK4
CHEMBL3589666	110	Dual specificity protein kinase CLK2
CHEMBL3589666	1500	Cyclin-dependent kinase 9
CHEMBL3589666	23	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589666	21	Dual specificity protein kinase CLK1
CHEMBL3589662	210	Dual specificity protein kinase CLK4
CHEMBL3589662	2100	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589662	600	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589674	220	Dual specificity protein kinase CLK1
CHEMBL3589674	79	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589674	3100	Cyclin-dependent kinase 9
CHEMBL3589674	1900	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589674	510	Dual specificity protein kinase CLK2
CHEMBL3589674	41	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589674	30	Dual-specificity tyrosine-phosphorylation regulated kinase 3
CHEMBL3589675	300	Dual specificity protein kinase CLK1
CHEMBL3589675	56	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589667	22	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589667	2300	Dual specificity protein kinase CLK4
CHEMBL3589671	3600	Dual specificity protein kinase CLK4
CHEMBL3589671	1300	Dual specificity protein kinase CLK2
CHEMBL3589671	2200	Cyclin-dependent kinase 9
CHEMBL3589671	180	Dual-specificity tyrosine-phosphorylation regulated kinase 1A
CHEMBL3589671	3400	Dual specificity protein kinase CLK1
CHEMBL3589671	4900	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589672	1400	Dual specificity tyrosine-phosphorylation-regulated kinase 1B
CHEMBL3589672	130	Dual-specificity tyrosine-phosphorylation regulated kinase 2
CHEMBL3589672	610	Dual specificity protein kinase CLK2
CHEMBL3589672	1100	Dual specificity protein kinase CLK4
CHEMBL3589672	1200	Dual specificity protein kinase CLK1
CHEMBL3589672	1500	Cyclin-dependent kinase 9
CHEMBL3589672	120	Dual-specificity tyrosine-phosphorylation regulated kinase 1A

Table S4 – Human targets for diclofenac (<1mM K_D/IC₅₀) from ChEMBL v23⁶

<u>ChEMBL Target ID</u>	<u>Target Name</u>	<u>Target Type</u>	<u>Value</u>	<u>Unit</u>	<u>Type</u>
CHEMBL1641347	Solute carrier family 22 member 6	SINGLE PROTEIN	4000	nM	IC50
CHEMBL2095157	Cyclooxygenase	PROTEIN FAMILY	500	nM	IC50
CHEMBL2157	Interleukin-8	SINGLE PROTEIN	8	nM	IC50
CHEMBL221	Cyclooxygenase-1	SINGLE PROTEIN	3	nM	IC50
CHEMBL230	Cyclooxygenase-2	SINGLE PROTEIN	5	nM	IC50
CHEMBL3194	Transthyretin	SINGLE PROTEIN	140000	nM	IC50
CHEMBL3194	Transthyretin	SINGLE PROTEIN	1000	nM	Kd
CHEMBL3253	Serum albumin	SINGLE PROTEIN	2454.71	nM	Kd
CHEMBL4029	Interleukin-8 receptor A	SINGLE PROTEIN	12	nM	IC50
CHEMBL5983	Aldo-keto reductase family 1 member B10	SINGLE PROTEIN	1900	nM	IC50

References

1. Dolinsky, T. J.; Nielsen, J. E.; McCammon, J. A.; Baker, N. A., PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. *Nucleic Acids Res* **2004**, 32 (Web Server issue), W665-7.
2. Ebejer, J. P.; Morris, G. M.; Deane, C. M., Freely available conformer generation methods: how good are they? *J Chem Inf Model* **2012**, 52 (5), 1146-58.
3. O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R., Open Babel: An open chemical toolbox. *J Cheminform* **2011**, 3, 33.
4. Goodsell, D. S.; Morris, G. M.; Olson, A. J., Automated docking of flexible ligands: applications of AutoDock. *J Mol Recognit* **1996**, 9 (1), 1-5.
5. Trott, O.; Olson, A. J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **2010**, 31 (2), 455-61.
6. Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P., ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res* **2012**, 40 (Database issue), D1100-7.